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Investigation of Carrier Oil Stabilized Superparamagnetic Iron oxide Nanoparticles and its Antibacterial Activity

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ABSTRACT

Superparamagnetic iron oxide nanoparticles (SPIONs) were synthesized by co-precipitation method. The present study aimed to understand the effect of carrier oil (Flaxseed Oil) on the precipitation and agglomeration behavior of the hydroxide precursors of the iron oxide nanoparticles. Kirby Bauer method was used to investigate the antibiotic sensitivity of carrier oil stabilized and uncoated SPIONs at 10 µL and 20 µL on gram-positive bacterium bacillus cereus (vegetative cell). The nanoparticles were characterized by X-ray diffraction method, FTIR analysis, particle size analyzer and Transmission Electron Microscopy. Structure of magnetite nanoparticles was confirmed by XRD analysis and the estimation of nanoparticle size was confirmed with TEM. The attachment of functional groups of oil was predicted using FTIR spectroscopy. This comparison study revealed that carrier oils stabilized iron oxide nanoparticles show more antibacterial activity than the bare iron oxide nanoparticles.

Keywords: Iron oxide nanoparticles, flaxseed oil, and bacillus cereus.

INTRODUCTION

The Iron oxide nanoparticles can be synthesized by co-precipitation method (Lan *et al*. 2007). The nanoparticles of iron oxide such as Fe_3O_4 and γ-Fe_2O_3 is very prominent material in biomedical applications[1-3]. The prevention of agglomeration is critical

factor during the synthesis and it can be controlled by appropriate stabilizer, but the stabilizing agent should ensure its function and not to have any toxicity to the nanoparticles. In this work flaxseed oil (Linseed oil) is used as a stabilizing agent. Flaxseed oil is a colourless to yellowish oil obtained from the dried, ripened seeds of the flax plant (Linum usitatissimum, Linaceae). Flax-based oils are sought after as food because of their high levels of poly unsaturated α-Linolenic acid (C18:3), which is a one form of omega-3 fatty acid. The other fatty acids Oleic acid (Monosaturated – Omega -6 - C18:1) and saturated Palmitic acid (C16:0) and stearic acid (C18:0) are also constituted[4-7]. Linseed oil is an edible oil marketed as a nutritional supplement[8]. Some studies suggest that α-linolenic acid (a substance found in flaxseed and flaxseed oil) may benefit people with heart disease[9-10].

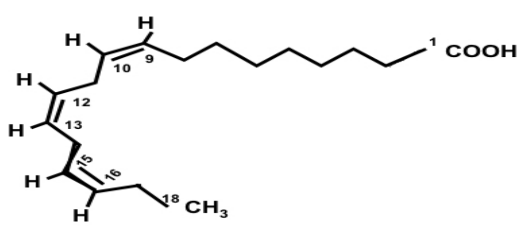

Fig 1. Structure of α-Linolenic acid

The antibacterial test on Bacillus cereus (B. cereus) was studied using this flaxseed oil stabilized iron oxide nanoparticles. Bacillus cereus is a bacterium that is commonly associated with large outbreaks of food borne illness. B.cereus has a wide distribution in nature, frequently isolated from soil and growing plants, but it is also well adapted for growth in the intestinal tract of insects and mammals (Stenfors Arnesen *et al*., 2008).

EXPERIMENTAL

All the chemicals were of analytical reagent grade and used without further purification. Ferrous sulphate ($FeSO_4$, 99%), ferric chloride ($FeCl_3$, 99%) and Sodium hydroxide (NaOH) were obtained from Merck (India) . Flaxseed Oil was obtained from Falcon Industries, India. The gram-positive bacterium bacillus cereus was purchased from Institute of Microbial Technology (Chandigarh, India), India.

Co-precipitation technique was adopted for making the SPIONs.100 ml of 0.4 mol/L solution $FeCl_3$ and 100 ml of 0.2 mol/L $FeSO_4$ were mixed and dissolved in deionized water. Then 2 mol/L of Sodium hydroxide was added into the above solution and the pH value was maintained between 10-11 with continuous stirring using a magnetic stirrer for 1 hour and a dark precipitation was formed. 5 ml of flaxseed oil was taken and heated to 80^0C in hot air oven and added in the precipitated solution for stabilization. The precipitated iron oxide particles were washed several times with double distilled deionized water and filtered. Finally it was dried at 150^oC for 2 hr and grinded to fine powder. Then the same procedure was followed for preparing bare iron oxide nanoparticles without adding of flaxseed oil.

X-Ray Diffraction (XRD) patterns were recorded with a Philips analytical X-ray diffractometer using CuKα radiation (λ= 1.5406 Å). FTIR spectra were performed and recorded with a Fourier transform infrared spectrophotometer of type Nicolet 870. TEM was recorded using Philips CM12 model. Particle size analysis was done by Malvern (U.K.) Make 2000E model.

Determination of antibacterial activity by well-diffusion method

Antimicrobial assay for the synthesized iron oxide nanoparticles were performed against gram positive Bacilli cereus by Kirby-Bauer disk diffusion method. The pure cultures of organisms were subcultured in Müller-Hinton broth at 121 deg C at 15 psi for 45 minutes under Autoclave. The medium is poured in to sterile petriplate and incibated at 37 deg for 24 hrs to check the plate sterility. The overnight grown Bacilli cereus ($4x10^9$) count a loop full of inoculum of was taken and swabbed on three dimensional lawn type on MHA plates. The sterile disc were coated with 10µg of each nanoparticle fix on the top surface of the medium. In another case 20µg of each nanoparticle was coated and fixed on the top surface of the medium. The plates were incubated at 37 deg C for 24 hrs and observed for every 4 hrs. It was observed that the zone of lysis was increasing as the incubation on prolonged incubation. After 24 hrs incubation the plates were examined for the appearance of zone of inhibition. The zone of inhibition was measured in mm and recorded.

RESULTS AND DISCUSSION

XRD analyses confirmed that the synthesized nanoparticles were magnetite (Fe_3O_4) as shown in figure2. The characteristic peaks were marked by their 2θ angles and compared with JCPDS data. The planes (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1) and (4 4 0) were observed for the corresponding angles of both the samples. The particle sizes were evaluated from the XRD data using the Debye – Scherrer equation and the average particle size of the flaxseed oil stabilized Fe_3O_4 nanoparticles is 32 nm and 52 nm for the uncoated sample.

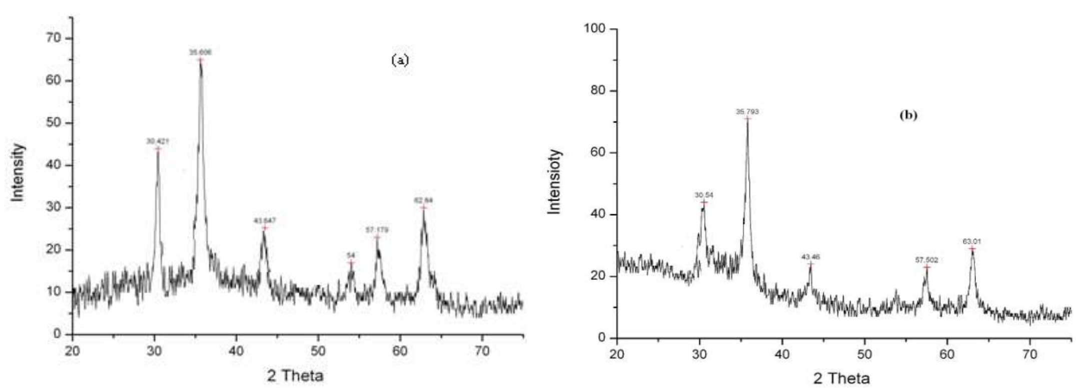

Fig 2. XRD patterns of Uncoated (a) and Flaxseed (b) SPIONs

The surface modification of the magnetic nanoparticles with their stabilization was confirmed by Fourier Transform Infrared Spectroscopy measurements (FTIR).

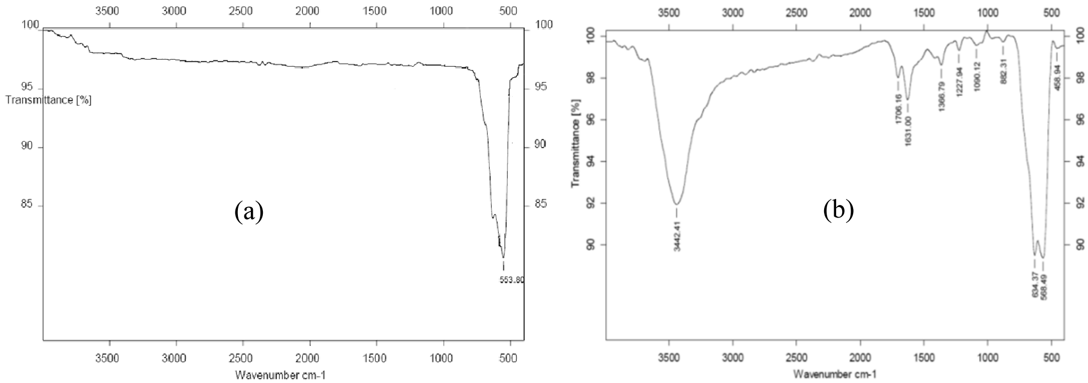

Fig 3. FTIR analysis of Uncoated (a) and Flaxseed (b) SPIONs

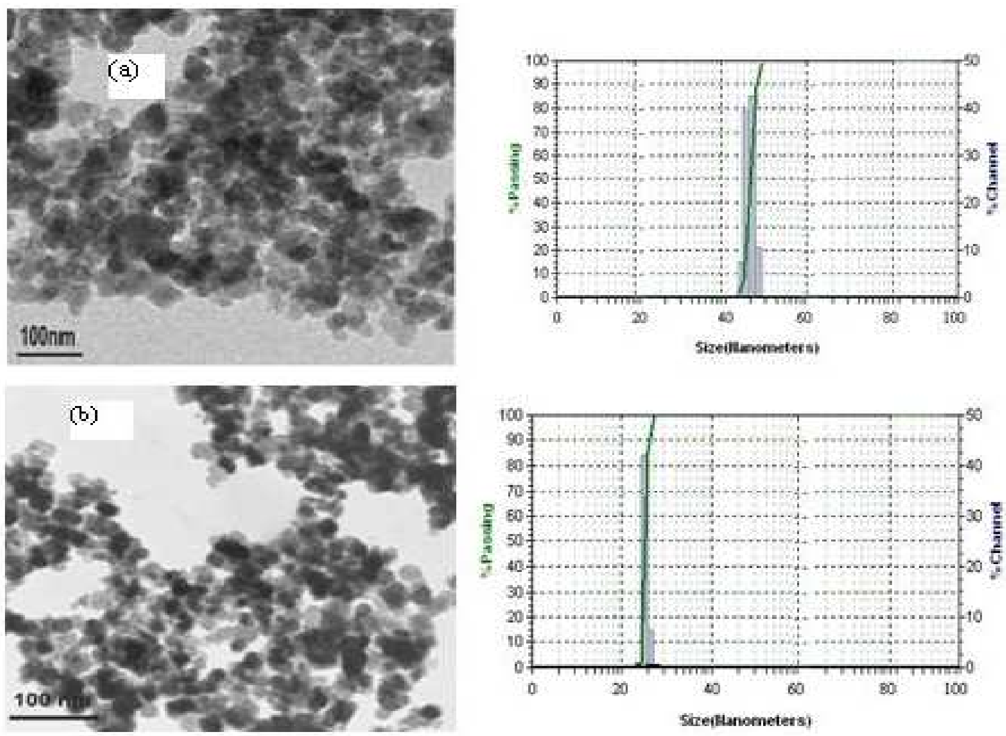

Fig 4. TEM photographs of Uncoated (a) and Flaxseed (b) SPIONs

The presence of absorption peaks in the region of wave numbers 550-630 cm^{-1} corresponding to the Fe-O vibration. The peaks at 3434 cm^{-1} in fig 2(a) and 3442cm^{-1} in fig 2 (b) were related to the vibrations of -OH and the peaks at 1707 cm^{-1}, 1629 cm^{-1}, 1706 cm^{-1} and 1631 cm^{-1} were due to the overlapping of the

absorption bands of the carboxyl groups and double bond of Oleic acid and α–Linolenic acid respectively. The other peaks observed in the region of 882 cm^{-1} -1366 cm^{-1} in sample (b) were due to the additional compounds (Polyphenols, Peroxides, Polycyclic Aromatic Hydrocarbons (PAHs),vitamin K and vitamin E) present in the oil.

The particle size and the morphology of magnetite NPs were observed by transmission electron microscopy (TEM). Fig.4 shows TEM micrographs of samples of uncoated and Flaxseed oil stabilized magnetite NPs and these images indicate that the particles are not aggregated and the mean physical sizes were almost the same as the values obtained by Debye – Scherer formula.

The antibacterial activity of iron oxide (Fe_3O_4) nanoparticles of uncoated (a) and flaxseed oil mediated samples were performed against gram- positive bacterium bacillus cereus (vegetative cell) at 10 µL and 20 µL concentrations using agar well diffusion method. The values of zone of inhibition (mm) of these nanoparticles were presented in the Table 1.

Table 1. Zone of inhibition (mm) of Uncoated (a) and Flaxseed (b) SPIONs

10µl of nanoparticle coated		
S. No	Nano particle	Zone of inhibition in(mm)
1	Flaxseed Oil Stabilized SPIONs	3.75
2	Uncoated SPIONs	1.5

20µl of nanoparticle coated		
S. No	Nano particle	Zone of inhibition in(mm)
1	Flaxseed Oil Stabilized SPIONs	8.5
2	Uncoated SPIONs	3.5

Fig 4. Zone of inhibition (mm) of Uncoated (a) and Flaxseed (b) SPIONs

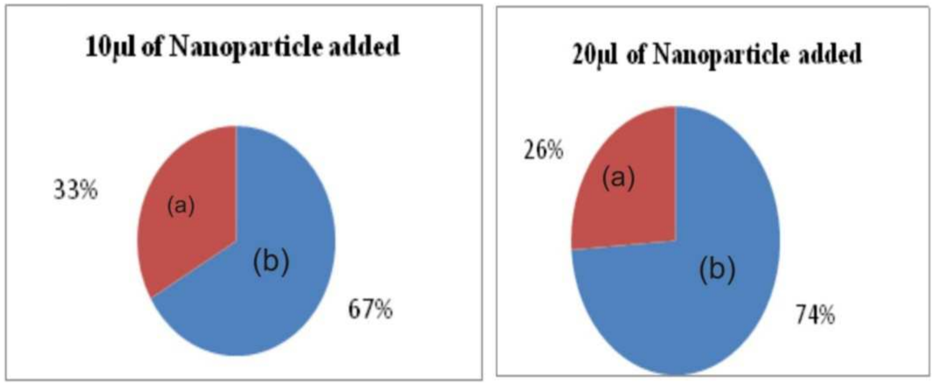

Fig 4. Pie chart : Zone of inhibition (mm) of Uncoated (a) and Flaxseed (b) SPIONs

The Figure 5 and 6 shows the zone of inhibition of bacterial growth on agar plates with two different concentrations of flaxseed oil stabilized and bare magentite nanoparticles. It was observed from the images and pie chart that the growth of bacteria was inhibited gradually with increase in concentration of Fe_3O_4 nanoparticles. Further the results clearly demonstrate that Flaxseed oil mediated Fe_3O_4 nanoparticles could promise a better antimicrobial agent than the bare Fe_3O_4 nanoparticles.

CONCLUSION

An Important need in the field of nanotechnology is the development of reliable and eco-friendly processes for synthesis of metal oxide nanoparticles. Here, we have reported a simple, eco-friendly and low-cost approach for preparation of magnetite nanoparticles by reduction of ferric chloride solution with a green method using flaxseed oil as the stabilizing and agent and compared with the uncoated Fe_3O_4. The characteristics of the obtained Fe_3O_4 nanoparticles were studied using FTIR, XRD and TEM techniques. The antibacterial effects of flaxseed oil stabilized Fe_3O_4 nanoparticles and bare Fe_3O_4 against gram- positive bacterium bacillus cereus (vegetative cell) were investigated. The results predicts that the flaxseed oil stabilized Fe_3O_4 nanoparticles were less size, more antibacterial activity than the bare iron oxide nanoparticles. The enhanced zone of inhibition was due to the presence of additional compounds in flaxseed oil and the carrier oils have antibacterial property.

REFERENCES

1. Jing Sun, Shaobing Zhou, PengHou, Yuan Yang, Jie weng, Xiaahong Li, Mingyuan Li., Synthesis and Characterisation of biocompatible Fe_3O_4 Nanoparticles, Wiley periodicals, *Inc. J. Biomed Mater Res*: 333 -341, (2007).
2. Seung-Jun Lee, Jong-Ryul Jeong, Sung-Chul Shin, Jin-Chul Kim, Jong-Duk Kim, Synthesis and characterization of superparamagnetic maghemite nanoparticles prepared by co- precipitation technique, *Journal of Magnetism and Magnetic Materials* 282,147–150 (2004).

3. Pan A, Yu D, Demark-Wahnefried W. Meta analysis of the effects of Flaxseed interventions on blood lipids, *American Journal of Clinical Nutrition,* 90(2): 288–297 (2009).
4. Angerer P, Von Schacky C. n-3 polyunsaturated fatty acids and the cardiovascular system. *Curr Opin Lipidol.* 11(1):57-63 (2000).
5. Balk E M, Lichtenstein A H, Chung M *et al*. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: A systematic review. *Atherosclerosis*. 189(1):19-30 (2006).
6. Barceló-Coblijn G, Murphy EJ, Othman R, Moghadasian MH, Kashour T, Friel JK. Flaxseed oil and fish-oil capsule consumption alters human red blood cell n-3 fatty acid composition: a multiple-dosing trial comparing 2 sources of n-3 fatty acid. *Am J Clin Nutr.* 88(3):801-9, (2008).
7. Barre D E, Mizier-Barre KA, Griscti O, Hafez K. Flaxseed oil supplementation increases plasma F1-phytoprostanes in healthy men. *J. Nutr.* 139 (10):1890-5, (2009).
8. Bassett CM, Rodriguez-Leyva D, Pierce GN. Experimental and clinical research findings on the cardiovascular benefits of consuming flaxseed. *Appl Physiol Nutr Metab.*34(5) : 965-74 (2009).
9. Bays HE. Safety considerations with omega-3 Fatty Acid therapy. *Am. J. Cardiol*. 99(6A):S35-43 (2007).
10. Yanglong Hou, Zhichuan Xu, and Shouheng Sun, Controlled Synthesis and Chemical Conversions of FeO Nanoparticles, *Angew. Chem. Int. Ed*., 46, 6329–6332X (2007).
11. R. Ye, C. Daraio, C.Wang, J. B. Talbot, and S. Jin., Room temperature solvent-Free Synthesis of Monodisperse Magnetite Nanocrystals, *Journal of Nanoscience and Nanotechnology* Vol.6, 852–856, (2006).